PCR protocol

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RBRC 11264 **Strain name** B6D2-Rasef<em1Osb>

Primer	primer_name	primer_seq	length		Reference
1	Inner Fw	CAATGAGTGTGACTCCGAGG	20		Depositor's primer
2		CTTCTTCATCCCTTATATCTGGAAC	25		RIKEN BRC
3	Outer Fw	AAACCTTAGCCTAGTAATGTTGG	23		Depositor's primer
4	Outer Rv	ACAGGATAATTACAAACTACAGG	23		Depositor's primer

Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

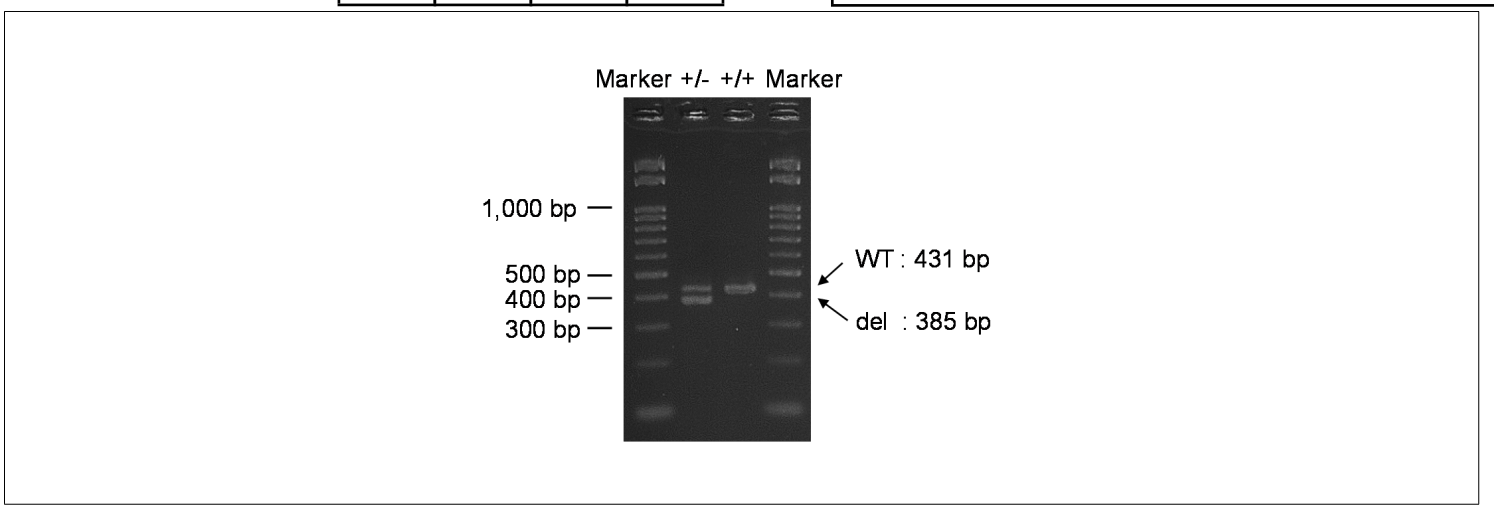
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	0.5				
primer 2	10 pmol/μl	0.5				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	60	* *Repeat for 30 cycles

Marker Gene Ladder 100 (Wako, Cat. 316-06951)

Gel 3% Nusieve in 0.5 X TBE (100 V, 50 min)



primer_set		product_size	product (allele type)	
primer 1	primer 2	431 bp	WT	+/+ (wild-type): 431 bp +/- (heterozygous): 431 bp and 385 bp
primer 3	primer 4	385 bp	del (em1)	

Comment Standard PCR

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.